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Involvement of NMDA in a Plasticity Phenomenon Observed in the Adult Frog Monocular Optokinetic Nystagmus

BLANDINE L. N. JARDON,* NICOLE BONAVENTURE*†

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The frog horizontal monocular optokinetic nystagmus (H-OKN) is asymmetrical, the reflex being evoked by a temporal–nasal (T–N) component, but not by a nasal–temporal (N–T) component. Coil recordings showed that, in adult animals, 8 days of monocular deprivation (by unilateral eyelid suture) provoked the appearance of a N–T component, the H-OKN becoming symmetrical, reacting for both directions of stimulation. This delay was shortened to 2 days following two successive unilateral pretectal administrations of NMDA or of LY 285 265, an NMDA agonist, the first 2 days of eyelid suture. The same results were obtained when chronic microinjections of NMDA or LY 285 265 were achieved, the frogs being maintained in total darkness during the week of eyelid suture. These data indicate that the plasticity phenomenon evidenced in the monocular frog H-OKN depends on the activation of the NMDA receptors of one pretectum. This activation was obtained either by a monocular light stimulation of 8 days duration, or by unilateral administration of drugs activating the NMDA glutamatergic pretectal system. In this last case, the light stimulation was no longer necessary. © 1997 Elsevier Science Ltd.

Plasticity Monocular H-OKN NMDA Pretectum Adult frog

INTRODUCTION

Glutamate, when activating NMDA receptors, has been strongly suggested to play a key role in visual plasticity in young animals. Involvement of the NMDA receptor in neocortical plasticity has been demonstrated by studies of photic responses in the visual system in mammals: application of the competitive NMDA antagonist 2-amino-5-phosphonovalerate (APV) prevented some cortical changes normally induced by monocular deprivation (Bear *et al.*, 1990; Rauschecker & Hahn, 1987); it disrupted segregation of retinogeniculate afferents in ferret lateral geniculate nucleus (Hahn *et al.*, 1991) and formation of a neuronal map in rat superior colliculus (Simon *et al.*, 1992). APV also disrupts ocular dominance columns in 3 eyed tadpoles (Cline *et al.*, 1987). These blocking effects of NMDA antagonists on visual responses appeared to be restricted to the critical period during the development of young animals affecting the adult visual system much less (Tsumoto *et al.*, 1987). However, in adult animals such as Xenopus, NMDA can restore the ability to realign the visual tectal map after alteration of an eye position, even after the end of the critical period (Scherer & Udin, 1989, 1991; Udin & Scherer, 1990).

In previous work, we have shown such a visual plasticity phenomenon in adult frog. In lower vertebrates, monocular horizontal optokinetic nystagmus (H-OKN) displays a directional asymmetry, the stimulation in the temporal–nasal (T–N) direction being always more efficient than the stimulation in the nasal–temporal (N–T) direction in evoking the reflex. In the frog, the H-OKN N–T component is even absent. However prolonged (8 days) monocular deprivation obtained by unilateral eyelid suture, abolished the monocular H-OKN asymmetry by provoking the appearance of a N–T component. The H-OKN slow phase velocity gain which was almost nil for a N–T stimulation 1 hr after eyelid suture, was significantly increased 8 days later, and no longer disappeared. The T–N component was not significantly modified, and no difference between the gain of both components was observed (Yücel *et al.*, 1990). Chronic administration of NMDA antagonists for the duration of deprivation prevented the appearance of a symmetrical monocular H-OKN in frogs: following repeated intraperitoneal injections of either MK 801, CGS 19755, or intrapretectal microinjections of APV, the N–T component did not appear and the H-OKN remained asymmetrical (Jardon & Bonaventure, 1992a).

In the frog, the pretectum and the nucleus of the basal

*Laboratoire de Neurosciences Comportementales et Cognitives, URA 1939 CNRS, 12 rue Goethe, 67000 Strasbourg, France.
†To whom all correspondence should be addressed [*Fax*: +33-08 35 84 42].

optic root (n BOR) were shown to be the mesencephalic structures responsible for OKN (Lazar *et al.*, 1983; Montgomery *et al.*, 1982; Yücel *et al.*, 1991). The pretectum receives the visual information directly from the contralateral retina and mainly from the central retina for the pretectal *nucleus lentiformis Mesencephali* (Montgomery *et al.*, 1981, 1985). The pretectal region is a very heterogeneous brain region, both anatomically and physiologically. It is located at the mesodiencephalic junction. The role of the *nucleus lentiformis Mesencephali* and of the postero-lateral thalamic nuclei is well established in the genesis of the H-OKN: a pretectal lesion entails the abolition of this reflex in the frog (Montgomery *et al.*, 1982), and the salamander (Manteuffel *et al.*, 1983). Physiological analysis of the responses of neurons of this area has shown that the pretectal cells have a preferential direction of discharge and are inhibited for stimulation in the opposite direction (Katte & Hoffmann, 1980). Moreover, it was strongly suggested that the retinal output to these mesencephalic nuclei was mainly glutamatergic (Nunez-Cardozo & Kamphuis, 1995; Hickmott & Constantine-Paton, 1993). A high density of NMDA receptor binding sites was evidenced in the frog pretectum (McDonald *et al.*, 1989).

All these data strongly suggested the involvement of NMDA receptor activation in building up the plasticity phenomenon observed in the frog oculomotor system. In the present paper, we have reported direct proof of the implication of NMDA in this compensatory process. Moreover, we have analyzed to what extent light stimulation of the open eye was necessary to evoke this plasticity phenomenon following an NMDA treatment. Indeed, it was previously shown (Yücel *et al.*, 1990) that, when frogs were put into total darkness during the week of unilateral eyelid suture, the monocular H-OKN remained asymmetrical, and the N–T component did not appear.

However, before studying the eventual involvement of the pretectal NMDA receptors in the plasticity phenomenon we have evidenced, the acute effects of the drug upon the directionality of the monocular H-OKN were analysed. In the first part of this report, monocular H-OKN was recorded before and after administration of NMDA or LY 285 265, an NMDA agonist (Schoepp *et al.*, 1991) into the pretectum or by systemic route. In the second part of this paper, the effects of successive injections of NMDA or of LY 285 265 on monocular H-OKN were analyzed during the week of monocular deprivation to see if the pretectal NMDA receptor activation could shorten the period of the build-up of the plasticity phenomenon. In the third part, frogs were daily treated with NMDA or LY 285 265 in total darkness during the week of "monocular deprivation", to see to what extent the effects of NMDA on the build-up of monocular symmetrical H-OKN depend on light stimulation.

METHODS AND TECHNIQUES

Monocular H-OKN was recorded in adult frogs (*Rana esculenta*) using the magnetic field search coil technique, in head restrained animals. Recordings were carried out before and after administration of the drug either intraperitoneally, into the occluded non-recorded eye,* or directly into the pretectum contralateral to the viewing eye. All animals underwent eyelid suture of the left eye under local anesthesia and were tested 1 hr later for control.

Stimulation

The frogs were placed in an optokinetic drum (300 mm in dia and 450 mm in height) with alternating black and white vertical stripes, distributed equally on its inner surface (10 mm wide). The drum was rotated clockwise and counter-clockwise at constant speed by means of an electronic control system. The range of the constant drum speeds used was between 1 and 9 deg/sec. Room illumination was kept constant at 80 lx at the level of the frog's eye.

Eye OKN recording

Eye H-OKN was recorded using a magnetic coil system as described by Koch (1977). One pair of coils (200 mm dia) carrying a current with a frequency of 50 kHz, generated a homogeneous magnetic field. These coils were mounted on a static platform. The sensing coil (1 mg, diameter of the copper wire 50 μm; inner diameter of the coil 2 mm, 70 turns, provided by Sokymat, Switzerland) was fixed on the eyeball in such a way to be oriented perpendicularly to the inter-aural axis, and was placed in the centre of the magnetic field. The voltage of the sensing coil, proportional to the sine of the horizontal angular displacement, was amplified, rectified, filtered and displayed on a paper recorder (BBC).

The system was calibrated before each recording by checking the linear relationship between the sensor coil angular displacement and the voltage induced in the sensor coil. The slow eye speed was measured using the cumulative curve of at least three successive slow phases at steady state, after elimination of the eye resetting fast phases. The slow phase velocity gain was defined as the ratio between the slow phase speed and the drum speed.

For purpose of data analysis, a Wilcoxon signed rank test was used.

In order to record monocular H-OKN, the lids of one eye were sutured, while the sclera of the other was exposed by removing the superior eyelid under local anesthesia (Cebesine, Chauvin Blache). The frog's head was immobilized by means of a nut, fixed on the skull and attached to a bar placed in the drum.

The sensing coil was secured (under local anesthesia) on the sclera with a drop of glue just before the experiment. The drugs were prepared as described below and administered either intravitreally, intraperitoneally or

*It was previously shown (Jardon & Bonaventure, 1992b) that drugs, when proved to cross the blood–brain barrier are conveyed by the bloodstream to central structures when injected into the closed eye, as after systemic injections.

directly into the pretectum, contralateral to the viewing eye.

Recordings were carried out before and after injection of the drug either into the closed eye (30 μl of solution), intraperitoneally (50 μl of solution) or directly into the pretectum (0.2 μl of solution). This last group of frogs underwent cannula implantation into the *nucleus lentiformis Mesencephali* of the pretectum.

Surgery

Frogs were prepared the day before the experiment under general anesthesia (MS 222 Sandoz). Experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609 EEC). A permanent stainless steel guide-cannula (o.d. 0.4 mm; i.d. 0.3 mm) was chronically implanted into the pretectum using a stereotactic procedure. The coordinates, according to the atlas of Wada *et al.* (1980) were A/P, 0.9 mm; M/L, 0.5 mm; D/V, 0.4 mm; with a head angle of 20 deg. The intersection of the anterior border of the left tectum and the sagittal midline was taken as reference.

The guide cannula, as well as the nut previously described, were anchored to the skull using retaining screws and dental acrylic cement. A stainless steel sterile mandrel (o.d. 0.27 mm) of the same length was inserted into the guide cannula to avoid obstruction.

Unilateral microinjections were performed in awake animals. A stainless steel injection cannula (o.d. 0.28 mm; i.d. 0.18 mm) was introduced into the guide cannula, so that it extended 0.1 mm beyond the tip of the guide cannula. The injection cannula was connected to 1 μl Hamilton microsyringe via polyethylene tubing filled with distilled water. It was filled with the drug by aspiration. A small air gap separated the two liquids. The drugs or the vehicle were injected in a volume of 0.2 μl over 20 sec. Movement of the air gap down the tubing was indicative of successful administration. The injection cannula was left in place for an additional 30 sec following drug administration. The mandrel was replaced into the guide cannula. The theory that a pretectal injection of saline has no effect upon the recorded OKN was tested.

Histology

To ascertain the injection site (Fig. 1), frogs were deeply anasthetized in MS222 following 8 days of post-injection survival time. After transcardiac perfusion with 0.9% saline followed by 4% formalin, brains were removed and placed into a 4% formalin solution. Paraffin-embedded brains were cut in 20 μm slices and processed with cresyl-violet stain. All the animals in which the cannula track could not be clearly localized were excluded from the study.

Drugs

NMDA (Sigma Chemical Co) was injected directly into the pretectum, since it does not cross the blood–brain barrier. LY 285 265 (D,L-tetrazol-5YL) (glycine) (Ely Lilly Company) (Schoepp *et al.*, 1991) is an NMDA agonist which crosses the blood–brain barrier; it was injected into the occluded eye, intraperitoneally or directly into the pretectum. The subtype of NMDA receptors it binds with is presently unknown (D. Schoepp, personal communication).

The drugs were respectively dissolved in phosphate buffer saline (PBS) 0.02 M (pH = 7.4) or in distilled water. The concentrations used were determined from the literature and from pilot studies.

The concentration of NMDA injected into the pretectum was 2.9 ng (10^{-4} M), and 0.29 ng (10^{-5} M). The concentrations of LY 285 265 injected into the

FIGURE 1. Photomicrograph showing a coronal section (20 μm) of the mesodiencephalic region of the frog brain. The tip of the cannula track represents the site of injection and is located medially to the anterior part of the optic tectum in the nucleus *Lentiformis Mesencephali*.

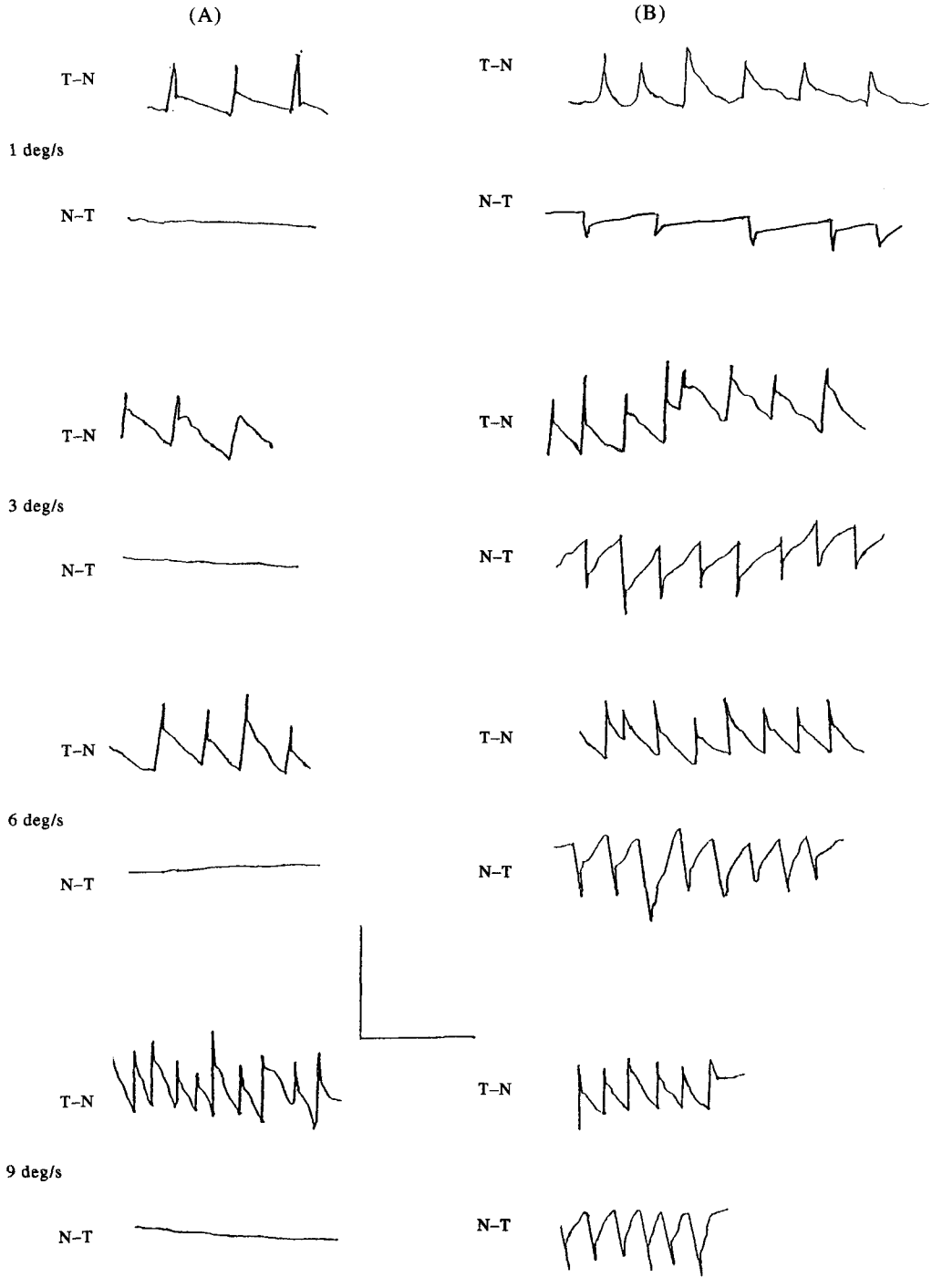

FIGURE 2. Coil recordings of monocular eye OKN evoked by four different constant drum speeds in a monocular viewing condition, before (A) and half an hour (B) following an injection into the closed eye of LY 285 265 (0.21 μg). The speed and the direction of the stimulus are indicated on the left of the recordings. Calibration: vertical bar, angular displacement of 10 deg; horizontal bar: 10 sec duration.

occluded eye were 21 μg (5×10^{-3} M), 2.1 μg (5×10^{-4} M), 0.21 μg (5×10^{-5} M), 21 ng (5×10^{-6} M); intraperitoneally, 3.5 μg (5×10^{-4} M), 0.35 μg (5×10^{-5} M), and into the pretectum 0.14 ng (5×10^{-6} M).

Procedure

Recordings began half an hour after intravitreal or intraperitoneal drug administration; they began immediately following intrapretectal drug injection in order to disregard the drug diffusion into cortical structures. Some recordings were made later to test the reversibility of the drug effect.

All animals underwent eyelid suture of the left eye under local anesthesia, and were tested 1 hr later for control.

Three series of experiments were conducted: in the two first series, all experiments were done in normal day–night illumination. In the first series, studying the acute

effects of NMDA, frogs were tested before and after administration of various concentrations of drug, injected either by systemic route ($n = 44$) or directly into the pretectum ($n = 35$).

In the second series of experiments, studying the role played by NMDA in the plasticity phenomenon, frogs were divided into four groups:

Group 1 or control group ($n = 13$) received no drug injection during the week of monocular deprivation.

Group 2 ($n = 13$) received daily an injection of PBS (phosphate buffer saline, 0.05, pH=7.4) during 8 days of monocular deprivation, either by intraperitoneal route ($n = 7$) or directly into the pretectum ($n = 6$). They were tested daily.

Group 3 ($n = 52$) frogs received an intraperitoneal injection of LY 285 265 (3.5 μg, $n = 20$ or 0.35 μg, $n = 32$) the first 2 days of the monocular deprivation week. They were tested daily.

Group 4 frogs received a unilateral microinjection of either NMDA (0.29 ng, $n = 21$) or LY 285 265 (0.14 ng, $n = 12$) into the pretectum contralateral to the open eye, the first 2 days of the monocular deprivation week. They were tested daily.

In the third series of experiments, frogs were put in total darkness the week of "monocular deprivation". They were divided into the same four groups:

Group 1 or control group ($n = 6$) received no drug during the week of monocular deprivation.

Group 2 ($n = 16$) received an injection of PBS daily, either by intraperitoneal route ($n = 6$) or directly into the pretectum ($n = 10$).

Group 3 ($n = 23$) received an intraperitoneal injection of LY 285 265 daily (0.35 μg, $n = 15$ or 3.5 μg, $n = 8$) during the 7 days of total darkness.

Group 4 ($n = 60$) received a unilateral microinjection of NMDA daily (0.29 ng, $n = 17$) or LY 285 265 (0.14 ng, $n = 25$; 1.4 ng, $n = 18$) into the pretectum contralateral to the open eye, during the 7 days of total darkness. Cannula implantation was conducted 1 day prior to placing the animals in total darkness.

Intraperitoneal and intrapretectal drug administration were carried out in weak red light.

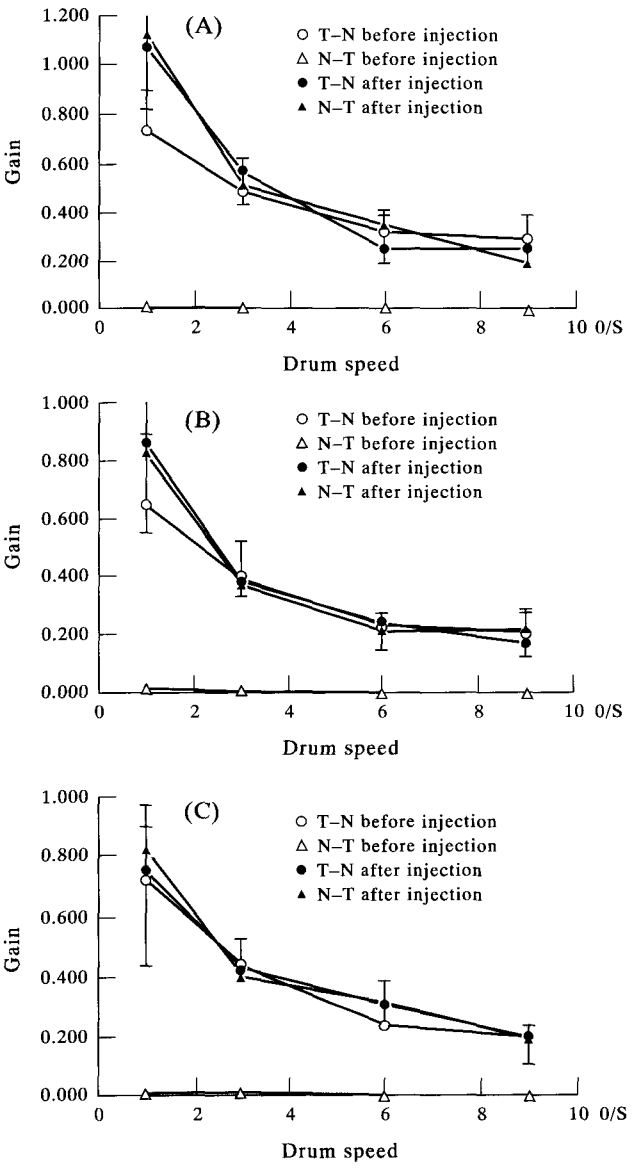

FIGURE 3. Means values of slow phase velocity gain of monocular eye OKN before (open symbols) and 30 min after injections of LY 285 265 (filled symbols) into the closed eye. (A) 21 μg, $n = 9$; (B) 2.1 μg, $n = 10$; (C) 0.21 μg, $n = 6$. The vertical bars indicate the standard deviation.

RESULTS

Control conditions

The monocular H-OKN was recorded in each animal, before injection, at four different drum speeds and in both directions of stimulation. In these conditions, the viewing eye predominantly followed the stripes moving in the T–N direction ($n = 79$). When the stimulation was applied in the N–T direction, no eye movement was detected, irrespective of the drum speed tested [Fig. 2(A)]. Injection of PBS did not change the OKN gain when administered either intraperitoneally ($n = 3$), into the occluded eye ($n = 3$), or into the pretectum ($n = 3$). Additionally, surgical cannula implanted into the pretectum did not modify the control OKN.

Monocular eye H-OKN following acute intraocular administration of LY 285 265 into the occluded eye

No spontaneous eye movement was observed after administration of LY 285 265, irrespective of the concentation used (21 μg: $n = 9$; 2.1 μg: $n = 10$; 0.21 μg: $n = 6$; 21 ng: $n = 2$).

The H-OKN recording started 30 min after drug injection [Fig. 2(B)]. For a T–N stimulation, no change was noted in the monocular H-OKN when compared to that recorded before injection for the three highest drum speeds tested [Fig. 3(A, B, C)]. The average velocity gain

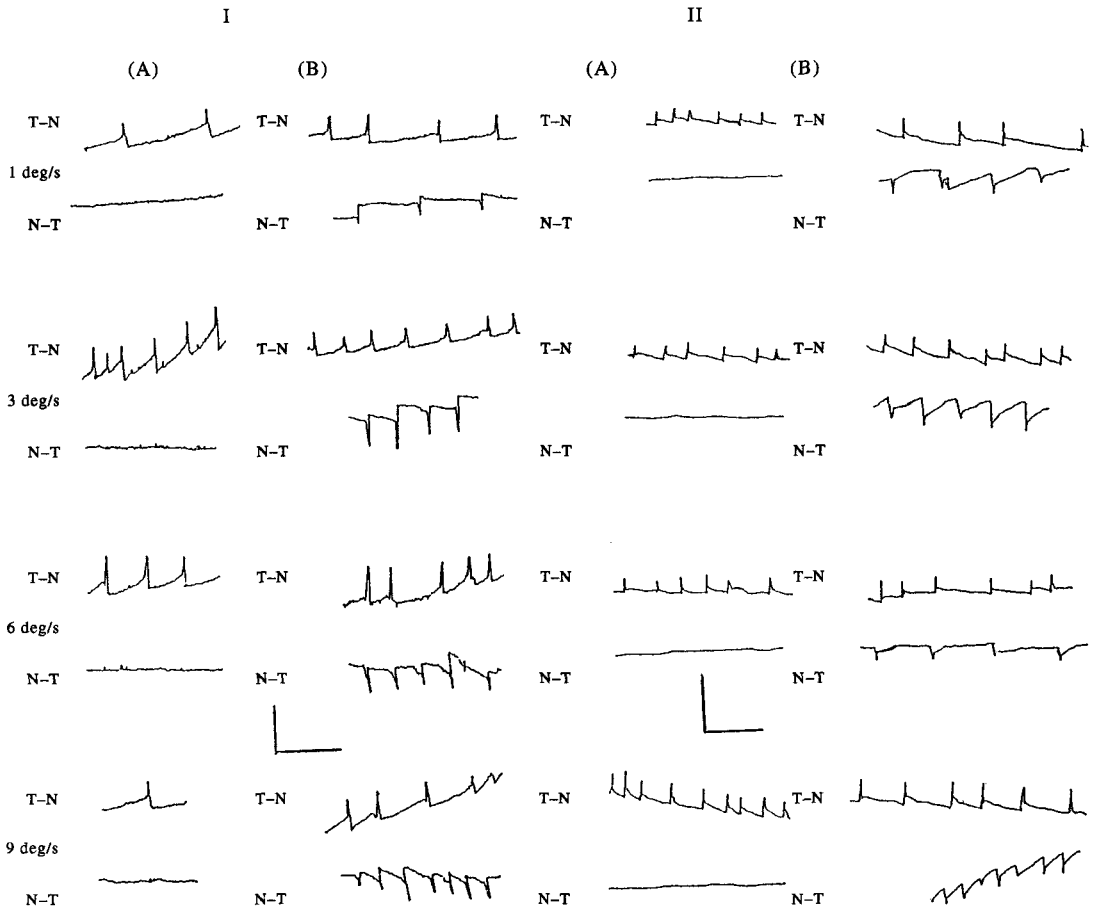

FIGURE 4. Coil recordings of monocular eye OKN evoked by four different constant drum speeds in a monocular viewing condition, before (A) and immediately following (B) a unilateral microinjection of either I: LY 285 265 (0.14 μg) or II: NMDA (0.29 ng) into the nLM. Calibration: vertical bar, angular displacement of 10 deg; horizontal bar, 10 sec duration.

did not change significantly from the control value. However, for the lowest drum speed (1 deg/sec) the velocity gain markedly and significantly increased ($P < 0.005$) for the two strongest drug concentrations [Fig. 3(A, B)]. In particular for the highest one, the velocity gain even exceeded 1 [Fig. 3(A)].

For N–T stimulation, frogs displayed an eye H-OKN with slow phases following the alternating black/white vertical stripes, and resetting fast phases which were not observed in control animals (Fig. 2). The average velocity gain which was null before injection increased for all drum speeds tested [Fig. 3(A, B, C)]. The H-OKN became symmetrical with identical T–N and N–T components. The difference between the velocity gain of H-OKN evoked by a T–N stimulation and that evoked by a N–T stimulation, which was significant before injection was no longer significant after LY 285 265 ($P > 0.2$) irrespective of the drum speed and the drug concentration used. Three hours after injection of LY 285 265, the N–T component was still present. This effect proved to be reversible, since 24 hr after injection, the N–T component has totally disappeared. The same results were obtained when LY 285 265 was injected by intraperitoneal route ($n = 11$).

Monocular H-OKN following intrapretectal acute microinjections of LY285 265 or NMDA contralaterally to the viewing eye

The effects of both drugs were identical: after administration of LY 285 265, 0.14 ng ($n = 8$) or NMDA, 0.29 or 2.9 ng ($n = 13$) frogs not only followed the alternating black/white vertical stripes in the T–N direction, but also those moving in the N–T direction [Fig. 4(I and II)].

Slow phases and resetting fast phases were observed for both directions of stimulation. The H-OKN recorded in the T–N direction was not modified compared to that recorded before injection, even at the lowest drum speeds, conversely to what was observed after intravitreal administration of LY 265 285. The slow phase velocity gain of the T–N component was not significantly modified compared to that of the control. However, there was a significant increase ($P < 0.005$) in H-OKN gain for the N–T stimulation at the four drum speeds tested for both drugs used. The monocular H-OKN became symmetrical with both components. [Fig. 5(A, B)].

At a lower concentration ($n = 5$) NMDA did not provoke an effect upon the monocular H-OKN, and the

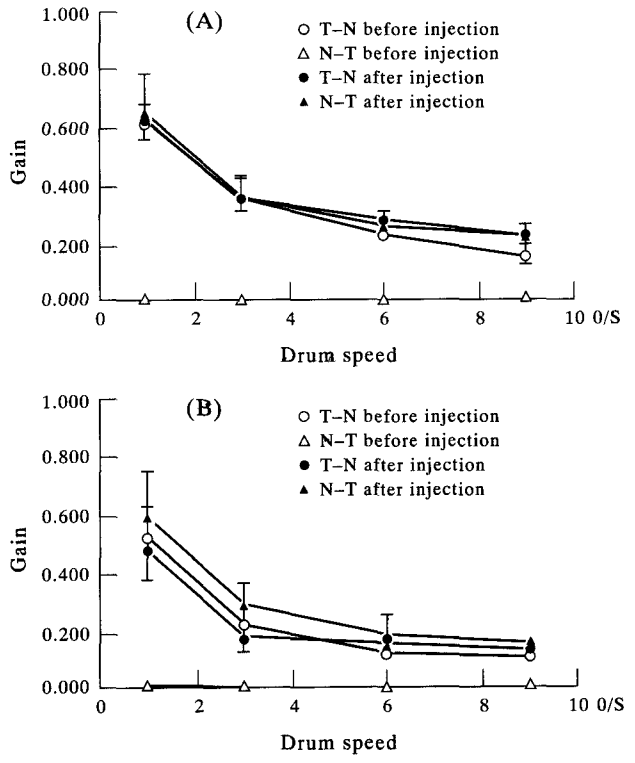

FIGURE 5. Mean values of slow phase velocity gain of monocular eye OKN, before (open symbols) and 10 min after unilateral microinjections (filled symbols) of (A): LY 285 265 (0.14 ng), $n = 8$; (B) NMDA (0.29 ng), $n = 13$ into the nLM contralateral to the open recorded eye. The vertical bars indicate the standard deviation.

N–T component did not appear. Frogs died at concentrations higher than those usually used.

Monocular H-OKN following drug injections during the monocular deprivation week in normal day–night illumination

Group 1 or control group ($n = 13$): frogs received no drug nor vehicle injection; 1 hr after unilateral eyelid suture, the frog's open eye followed predominantly the stripes moving in the T–N direction, and no eye movement could be detected when the stimulus was applied in the N–T direction, as mentioned above [Fig. 6(A)].

After 8 days of monocular deprivation, frogs displayed a monocular eye OKN when stimulated in the T–N direction, but also when stimulated in the N–T direction. The slow phase velocity gain which was almost nil for an N–T stimulation increased significantly and no difference between both T–N and N–T components was observed, as has been previously demonstrated (Yücel *et al.*, 1990).

Group 2 ($n = 13$): Following a week of monocular deprivation with daily intraperitoneal ($n = 7$) or intrapretectal ($n = 6$) administration of PBS, frogs followed the stripes moving in the T–N as well as in the N–T direction. Thus, chronic injections of PBS did not prevent or improve the appearance of the N–T component during the 8 days of unilateral eye deprivation [Fig. 6(B)]. The difference between the slow phase velocity gain of the OKN evoked by a T–N stimulation and that evoked by a N–T stimulation was no longer significant ($P > 0.3$) for all drum speeds used [Fig. 7(A)].

Group 3 ($n = 52$): On the first 2 days of visual deprivation frogs received an intraperitoneal injection of LY 285 265 at a concentration of 0.35 μg ($n = 32$) or 3.5 μg ($n = 20$). They were tested daily during the experimental week.

After the first injection of LY 285 265, frogs displayed a symmetrical monocular OKN with almost identical T–N and N–T components as was seen previously. The disinhibitory effect upon the N–T component lasted at least 4 hr; it was totally reversible: 24 hr after injection the N–T component had totally disappeared. At this moment, 24 hr following the first injection, a second injection of LY 285 265 at the same concentration was carried out and monocular OKN was again recorded. The N–T component reappeared with the same characteristics as those observed before, in particular, the velocity gain was again identical to that measured from the T–N component. However, in contrast to what was observed after the first injection, the N–T component no longer disappeared (without complementary injection). The monocular OKN recorded on the following days still displayed an N–T component, identical to the T–N component, the H-OKN remaining symmetrical [Figs 7(B) and 8(A)].

The same results were obtained with both concentrations of LY 285 265.

Group 4: Each of the first 2 days of visual deprivation, frogs received a unilateral drug administration of NMDA (0.29 ng, $n = 21$) or LY 285 265 (0.14 ng, $n = 12$) into the pretectum contralateral to the open eye.

Frogs were tested immediately after administration of the drug: as seen above, they displayed a symmetrical OKN with both identical components. However, the N–T component disappeared as soon as the drug effect vanished. Following the second intrapretectal administration of the drug, however, the N–T component reappeared with the same characteristics as those of the T–N component which remained identical to that recorded in the control before injection [Fig. 8(B)]. The monocular H-OKN was symmetrical. In this case also, the H-OKN recorded following an N–T stimulation did not disappear on the following days, without adding any supplementary drug injection.

Thus, following two successive injections of NMDA or NMDA agonist (over a period of 24 hr), the plasticity phenomenon seen after a week of monocular deprivation in control animals is built up earlier, only 2 days after eyelid suture.

We wanted to know the optimal duration between both injections, in order to obtain a permanent N–T component when LY 285 265 (0.35 μg) was intraperitoneally injected. The results are shown in Table 1.

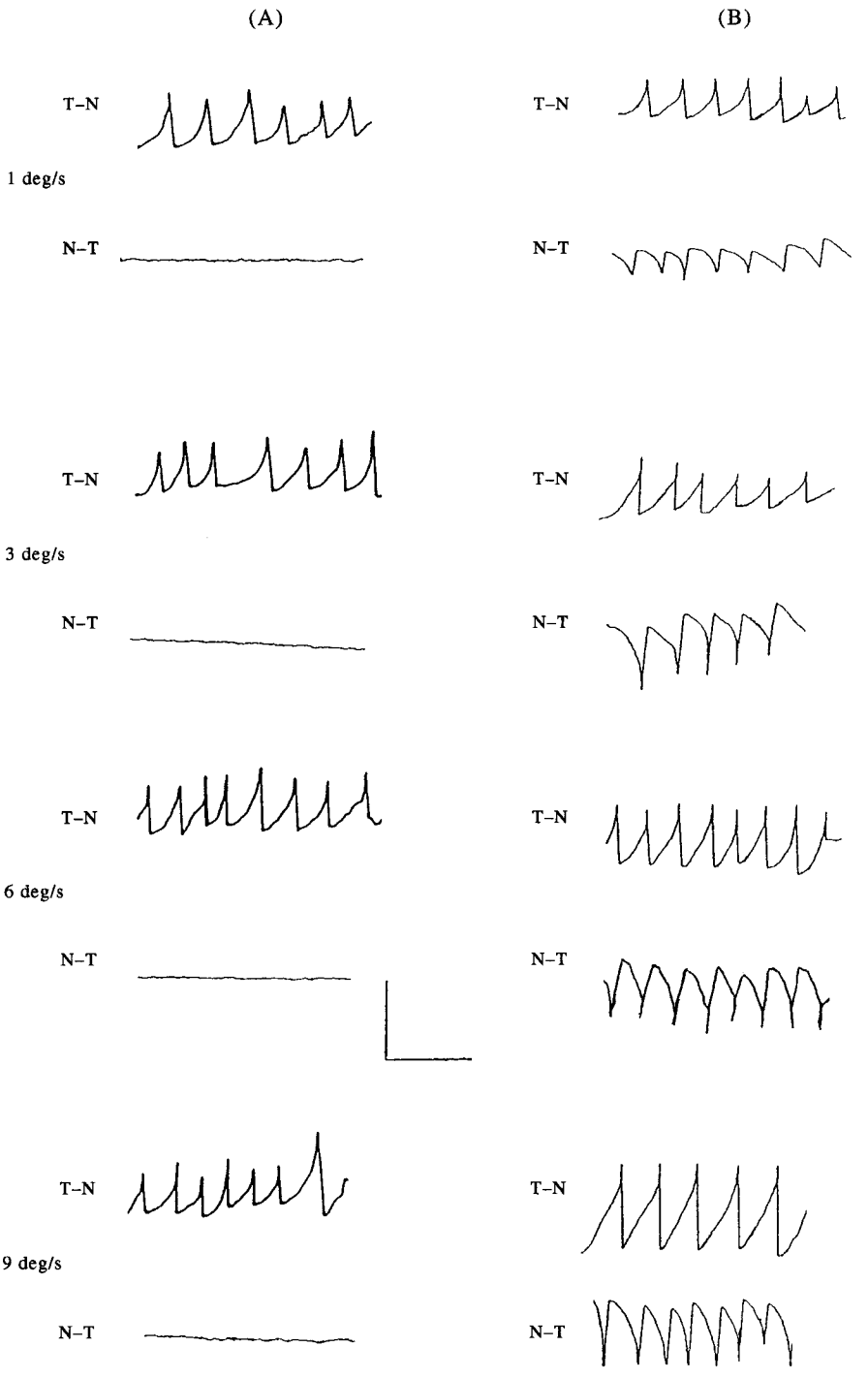

FIGURE 6. Coil recordings of monocular H-OKN evoked by four different constant drum speeds (A) 1 hr after monocular eyelid suture; or (B) following 8 days of monocular deprivation with daily intraperitoneal injection of PBS. Calibration: vertical bar, angular displacement of 10 deg; horizontal bar, 10 sec duration.

Thus, to obtain a durable and permanent N–T component, the delay between both injections could be from 3 to approx. 48 hr.

Monocular H-OKN following daily drug injections in animals maintained in total darkness for 1 week

In order to study the role of light stimulation in these plasticity phenomena, the same experiments as those conducted in normal illumination were carried out in total darkness for 1 week.

Group 1 or control group ($n = 6$): Frogs received no drug nor vehicle injection; after 8 days of monocular eyelid suture, frogs displayed an asymmetrical H-OKN with a T–N component identical to that of controls.

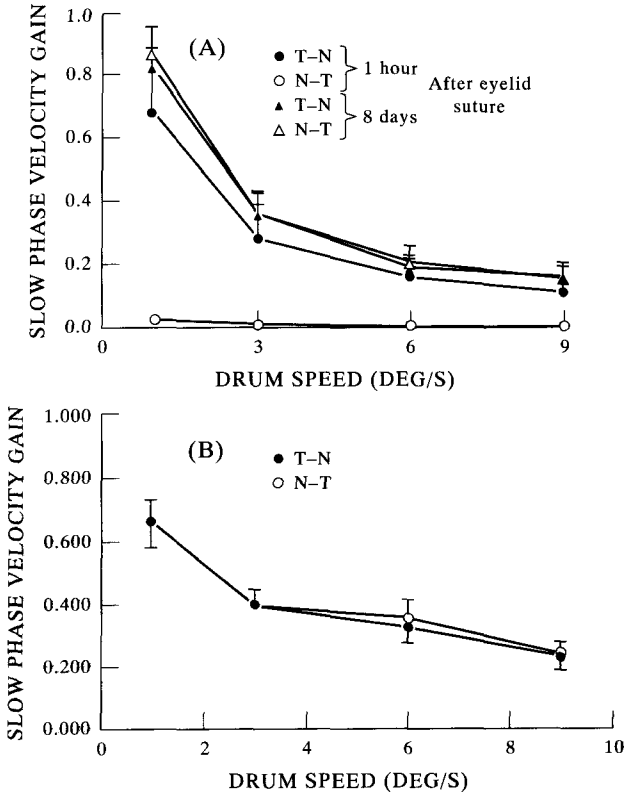

FIGURE 7. Mean values of slow phase velocity gain of monocular H-OKN (A) 1 hr (circles) or 8 days (triangles) after unilateral eyelid suture. (B) Three days after unilateral eyelid suture, the frogs having undergone two daily intraperitoneal injections of LY 285 265 (0.35 μg). Filled symbols, slow phase gain for T–N stimulation; open symbols, slow phase gain for N–T stimulation. The vertical bars indicate the standard deviation.

The N–T component did not appear. This observation confirms a previous one (Yücel *et al.*, 1990).

Group 2: Frogs received either an intraperitoneal ($n = 6$) or an intrapretectal ($n = 10$) administration of PBS daily. Under these conditions, the N–T component did not appear, the H-OKN recorded following a T–N stimulation was identical to that recorded in normal conditions of illumination. The H-OKN remained asymmetrical [Fig. 10(D)].

Group 3: These frogs received an intraperitoneal administration of LY 285 265 (3.5 μg, $n = 8$; 0.35 μg, $n = 15$) daily during the 7 days of total darkness. They were tested in the optokinetic drum 10–15 min after being brought out from darkness. The animals displayed an H-OKN for a T–N stimulation, but the N–T stimulation was unable to provoke any nystagmic response. The animals were then placed into a day–night illumination for 24 hr, without receiving any other drug infusion. The H-OKN recorded at this moment displayed a T–N component identical to the control; an N–T component appeared with the same characteristics as those of the T–N component. The monocular H-OKN became symmetrical [Fig. 9(A)] and remained symmetrical for many days later. (Animals were not tested beyond 15 days.) The velocity gain was identical for both components [Fig. 10(C)].

In some experiments ($n = 8$, LY 285 265 0.35 μg) animals were maintained for only 4 days in total darkness: results were analogous to those described above, when they remained for 7 days in total darkness, i.e., symmetrical H-OKN was recorded when animals were placed for 24 hr in day–night illumination.

Group 4: Animals received a unilateral microinjection of either NMDA (0.29 ng, $n = 17$) or LY 285 265 (1.4 ng, $n = 18$; 0.14 ng, $n = 25$) into the pretectum contralateral to the open eye. This treatment was carried out during 7 days in total darkness. Frogs were then tested in the optokinetic drum 10–15 min after being brought into the light. Animals immediately displayed a symmetrical H-OKN with both identical T–N and N–T components as soon as they were placed in the moving drum [Fig. 9(B)]. The difference between the slow phase velocity gain of the H-OKN evoked by a T–N stimulation and that evoked by an N–T stimulation was no longer significant ($P > 0.3$) for all drum speeds [Fig. 10(A, B)]. The effects of both drugs were identical, but it should be noted that at the lowest drum speed (1 deg/sec) the gain was increased up to 1 for the T–N component following NMDA administration.

The presence of the N–T component for at least 15 days was checked (the test was not carried out beyond this time). In some experiments ($n = 12$), frogs were maintained for only 4 days in total darkness: results were analogous to those described above, when animals were maintained for 7 days in total darkness.

Thus, in total darkness, frogs received daily either:

(1) a unilateral injection of NMDA or NMDA agonist into the pretectum contralateral to the open eye for 4 or 7 days: as soon as the animal was brought into light, the recorded monocular H-OKN was immediately symmetrical and the N–T component no longer disappeared.
(2) or an intraperitoneal (symmetrical) injection of LY 285 265 for 7 or 4 days: as soon as the animal was brought into the light, the monocular H-OKN proved to remain asymmetrical, only the T–N stimulation was able to provoke the reflex. However, if the frog was placed for 1 day in normal day–night illumination, the N–T component appeared and no longer disappeared.

DISCUSSION

In monocular viewing conditions, frogs display an asymmetrical horizontal OKN, reacting only to stimulation in the T–N direction and not to stimulation in the opposite direction. However, our results demonstrate that systemic or intrapretectal administration of respectively NMDA agonist (LY 285 265) or NMDA itself, causes the

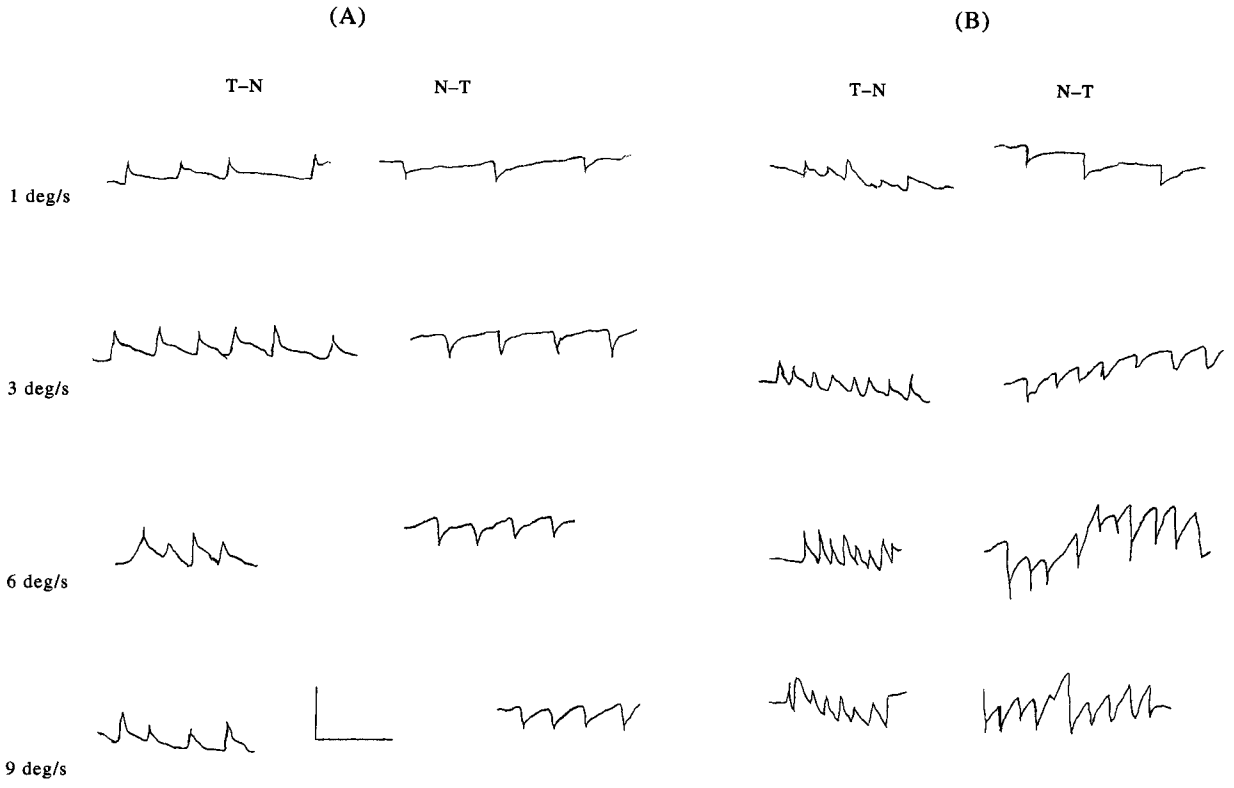

FIGURE 8. Coil recordings of monocular H-OKN evoked by four different constant drum speeds, 3 days after monocular eye deprivation, the frog having undergone two daily (A) intraperitoneal injections of LY 285 265 (0.35 μg); (B) unilateral microinjections of LY 285 265 (0.14 ng) into the pretectum contralateral to the viewing eye. Calibration: vertical bar, angular displacement of 10 deg; horizontal bar 10 sec duration.

appearance and the increase of a N–T component, thus abolishing the asymmetry of the reflex.

The systemic injection of LY 285 265 does not enable localization of its site(s) of action. However, the similar effect of the drug, when injected either by general route or directly into the pretectum, leads us to conclude that NMDA receptors involved in this process must be localized at least partially in the pretectum. The high density of NMDA binding sites found in this structure (McDonald *et al.*, 1989) supports this conclusion.

We have previously shown that, in frogs, prolonged unilateral eyelid suture (8 days) abolished monocular H-OKN asymmetry, provoking the progressive appearance of an N–T component which does not exist in control animals. Chronic administration of NMDA antagonists during the week of monocular deprivation prevented the abolition of monocular H-OKN asymmetry: the N–T component did not appear, remaining almost nil (Jardon & Bonaventure, 1992a). Conversely, the results of the present paper indicate that the administration of NMDA, or of a potent agonist, such as LY 285 265, on the first 2 days of monocular deprivation, reduces the delay of the appearance of an N–T component and at the same time abolishes the monocular H-OKN asymmetry. One of the most interesting points is that the monocular H-OKN remains symmetrical the following days, without additional drug administration. Thus, NMDA receptor activation seems to reduce the duration of the compensatory phenomenon development seen in control animals, during the week of monocular deprivation. Therefore, glutamate, when acting through NMDA receptors, appears to be involved in the mechanism underlying such a plasticity phenomenon observed in adult animals. This has already been shown in another model of visual

TABLE 1.

Delay between both injections	Number of frogs	Duration of the observations of a stable N–T component
3 hr	26	17 days at least*
24 hr	17	9 days at least*
48 hr	5	11 days at least
5 days	5	N–T appeared and was maintained only the day after each injection, after that it vanished

*For technical reasons, it was not possible to test the frog beyond this period. Delay between both intraperitoneal injections of Ly 285 265 (0,35 μg) necessary to obtain a stable N–T component.

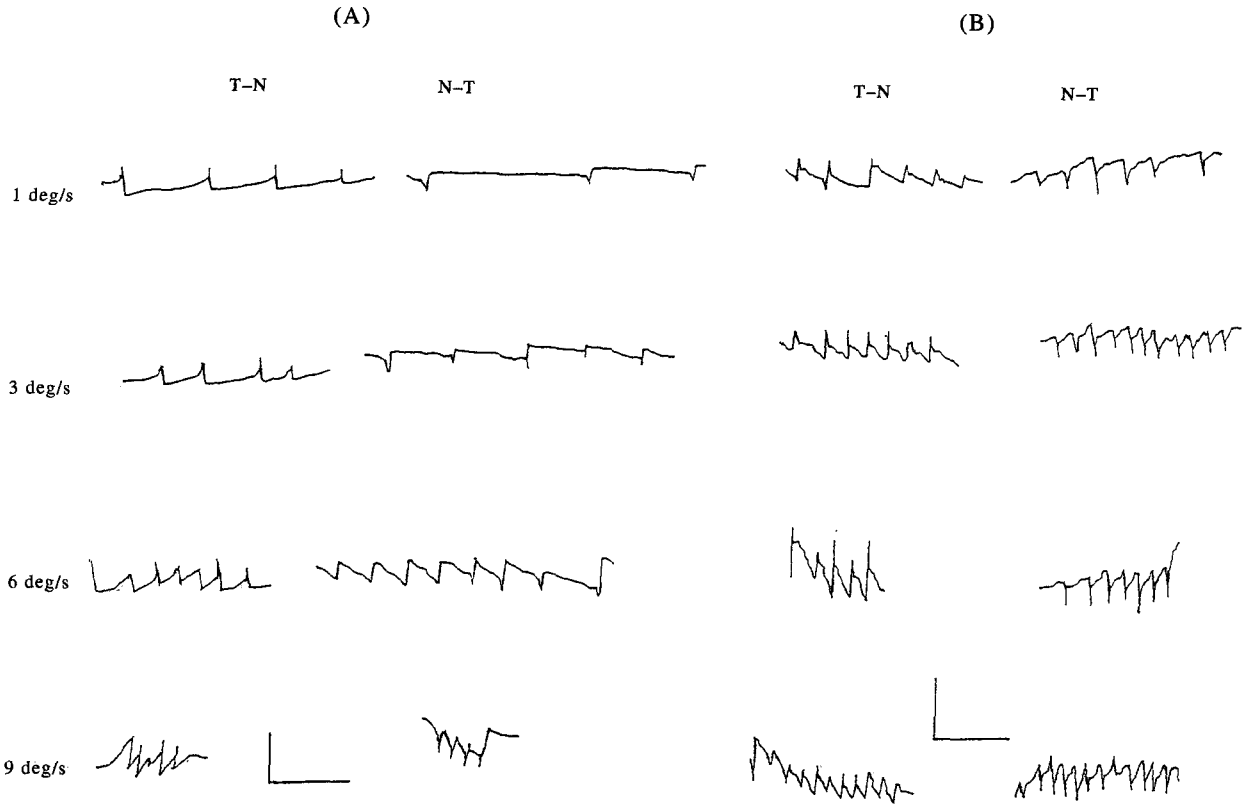

FIGURE 9. Coil recordings of monocular eye H-OKN evoked by four different constant drum speeds, the frog having undergone seven daily successive (A) intraperitoneal injections of LY 285 265 (0.35 μg) in total darkness, recordings being taken 24 hr after the animals were again placed in normal day–night illumination; (B) unilateral microinjections of LY 285 265 (0.14 ng) into the pretectum contralateral to the open eye in total darkness, recordings being achieved immediately (10–15 min) after the animals were again placed in normal day–night illumination. Calibration: vertical bar, angular displacement of 10 deg; horizontal bar, 10 sec duration.

plasticity in frogs (Udin & Scherer, 1990). However, why are two successive drug administrations necessary to provoke the stability of this compensatory process? According to Skangiel-Kramska (1988), the activation of the NMDA receptors must reach a critical threshold to bring about these plasticity phenomena.

Experiments carried out in total darkness have shown that the N–T component of the monocular H-OKN does not appear in control animals. However, if, in these conditions of light deprivation, frogs received an injection of either NMDA or an NMDA agonist daily, the results differ according to the mode of drug administration: when the drug is "asymmetrically" injected, i.e., into the pretectum contralateral to the open eye, frogs display an N–T component in the monocular H-OKN triggered by the open eye as soon as they are brought into the light. When the drug is "symmetrically" injected by intraperitoneal route, the H-OKN triggered by the open eye does not display an N–T component, remaining asymmetrical when frogs are brought out of darkness. But if the open eye is then normally light-stimulated for some hours, the N–T component appears and will no longer disappear for at least 15 days. These experiments indicate that unilateral pretectal injections of NMDA are sufficient to obtain a permanent symmetrical monocular H-OKN, light stimulation being unnecessary to build up the plasticity phenomenon. On contrast, the symmetrical injection of the drug in total darkness is not able to provoke alone, the appearance of the N–T component. A unilateral light stimulation is required in addition, for a short period, to provoke a symmetrical monocular H-OKN.

Thus, to build up the plasticity phenomenon we have seen, a unilateral NMDA pretectal activation is required. It is obtained by direct unilateral administration of NMDA or an NMDA agonist into the pretectum; it can also be obtained by monocular illumination during 8 days. However, in this case, a previous symmetrical drug administration, even in total darkness reduced the period of the build-up of the symmetrical monocular OKN. Therefore, maintaining adult animals in total darkness with NMDA administration, seems to reduce the period of build-up of this plasticity phenomenon. It has already been shown (Fox *et al.*, 1991; Williams *et al.*, 1996) that dark rearing prolongs plasticity and delays the loss of NMDA receptor function in the visual system, but only in young animals.

Now, the problem remains to determine what mechan-

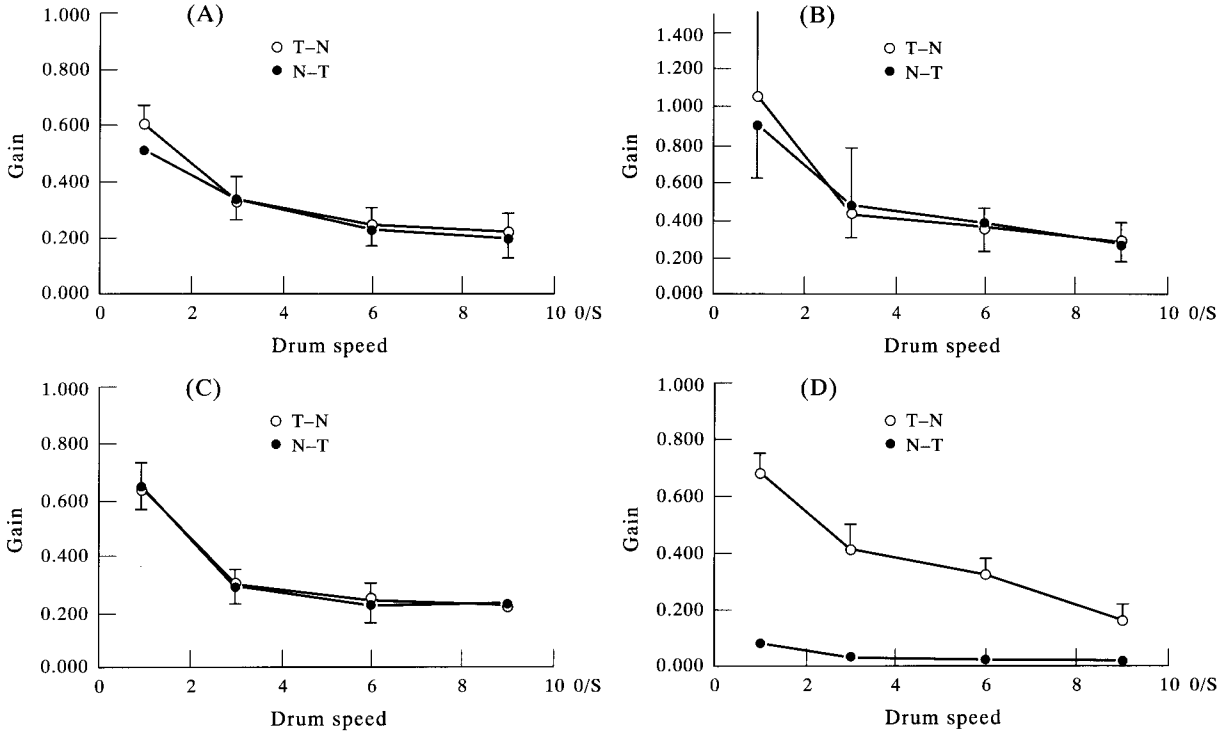

FIGURE 10. Mean values of slow phase velocity gain of monocular eye H-OKN following 7 days of total darkness in four different experimental conditions, frogs having undergone seven daily unilateral microinjections of drugs into the pretectum contralateral to the open eye. (A) LY 285 265 (0.14 ng); (B) NMDA (0.29 ng); or (D) PBS. Frogs have undergone 7 daily intraperitoneal administration of (C) LY 285 265 (0.35 μg). Recordings were achieved immediately (10–15 min) after the animals were again placed in normal day–night illumination (A, B, D) or 24 hr later (C).

ism underlies the effect of NMDA receptor activation. It seems that the compensatory process observed in monocular H-OKN occurs too quickly to be related to anatomical changes. Nevertheless, this hypothesis cannot be totally dismissed: indeed, it was observed that chronic treatments with low levels of NMDA entail fine synaptic structural alterations and local synaptic rearrangements within the retino-tectal neuropile in developing frog (Yen *et al.*, 1993).

The activation of NMDA receptors was found to initiate long-term potentiation (LTP) of synaptic transmission (Collingridge & Bliss, 1987); it is also involved in the plasticity of visual responses (Bear *et al.*, 1990; Iwakiri & Komatsu, 1993; Udin & Scherer, 1990; Cline *et al.*, 1987; Udin *et al.*, 1992). The question remains whether the long-term adaptative visual modifications observed in monocular H-OKN characteristics are controlled by a mechanism analogous to that underlying LTP. Recent studies lend support to a role for Ca^{2+} influx through NMDA receptors in synaptic plasticity (Perkel *et al.*, 1993): as for LTP, the intracellular influx of Ca^{2+} could serve as a second messenger to trigger enzyme translocation or activation, protein-kinase C playing an essential role in this mechanism. This enzymatic cascade once induced, could provoke durable metabolic and/or molecular modifications in the cell.

It has previously been shown that pretectal GABAergic and cholinergic system are also involved in these compensatory processes. (Yücel *et al.*, 1990, 1991; Jardon & Bonaventure, 1992b). The administration of the $GABA_A$ agonist THIP transiently abolished the monocular H-OKN symmetry by suppressing the N–T component which appeared after a week of unilateral occlusion. In contrast, intrapretectal administration of $GABA_A$ antagonists induced the appearance of an N–T component in the frog's monocular H-OKN. In the same way, the N–T component was also induced by administration of ACh muscarinic agonists, while atropine, an ACh muscarinic antagonist transiently suppressed the N–T component which appeared following prolonged unilateral visual deprivation. Thus, the plasticity phenomenon observed in monocular frog's H-OKN seems to require a reduction of GABAergic inhibition and a concomitant activation of muscarinic cholinergic transmission occuring progressively in the pretectum. Interactions between GABAergic and cholinergic mechanisms were demonstrated at the pretectum level (Bonaventure & Jardon, 1992). But are these alterations in GABAergic and cholinergic transmissions linked to NMDA receptor activation?

In other cortical structures it was shown that carbachol can potentiate NMDA responses in the rat hippocampus (Harvey *et al.*, 1993) and muscarinic receptor activation can induce a long-lasting enhancement of Purkinje cell responses to glutamate (Andre *et al.*, 1993). Moreover, intact cholinergic input was necessary to provoke the

shift of ocular dominance after monocular visual deprivation during the critical period in kittens (Bear & Singer, 1986).

On the other hand, the induction of LTP in the visual cortex of the rat requires both activation of NMDA receptors and the concomitant reduction of GABA inhibition (Artola & Singer, 1987); a GABAergic mechanism inhibits the formation of LTP in the rat superior colliculus (Hirai & Okada, 1993). These data indicate that GABAergic mechanisms might control the NMDA-mediated processes. Moreover, it was recently shown that GABAergic receptor responses can be suppressed by NMDA application in hippocampal neurons isolated from the adult guinea-pig (Chen & Wong, 1995). On the other hand, it has been suggested that the directionality of monocular eye H-OKN was determined by the directional selectivity of pretectal cells: many units recorded in the frog pretectum are stimulated in the T–N direction and inhibited by stimulation in the N–T direction (Katte & Hoffmann, 1980; Cochran *et al.*, 1984), the inhibition of unit activity during the N–T stimulation resulting in GABAergic activation. By analogy with the results of Chen and Wong, it is tempting to think that the pretectal NMDA receptor activation could suppress the GABAergic inhibition at this level. Thus, it is suggested that NMDA receptor activation requires ACh stimulation and entails a reduction of GABAergic inhibition.

According to these data we propose the following hypothesis: the chronic activation of pretectal NMDA receptors causes modifications resulting in significant functional reorganization in monocular H-OKN. This activation appears to be facilitated by ACh inputs acting on pretectal muscarinic receptors (Jardon *et al.*, 1991), and seems simultaneously inhibit GABAergic terminals found in this structure (Yücel *et al.*, 1988).

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